Attributes of Bean Yellow Mosaic Potyvirus Transmission from Clover to Snap Beans by Four Species of Aphids (Homoptera: Aphididae)

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ABSTRACT After characterization of the natural spread of necrosis-inducing Bean yellow mosaic potyvirus (family *Potyviridae*, genus *Potyvirus*, BYMV^N), nonpersistently transmitted from clover, *Trifolium repens* L., to an adjacent field of snap bean, *Phaseolus vulgaris* L., in western Oregon, we established a study site enabling us to investigate the virus reservoir, to observe en masse transmission of BYMV^N to bean plants, and to identify aphid species associated with virus spread. Colonies of *Myzus persicae* (Sulzer), *Acyrthosiphon pisum* (Harris), and *Aphis fabae* Scopoli associated with virus spread were established in an insectary and shown to vector this virus. Although *Nearctaphis bakeri* (Cowen) comprised 68% of aphid alatae taken from bean leaves during virus spread, we were unable to show that this species could vector the virus by using the same methods that were successful for the other species. Instead, we found that when two distinct *N. bakeri* colonies unexpectedly emerged from the roots of *T. repens* BYMV^N source plants (WZwc #6 and #11) that were present in the laboratory (insectary), these aphids transmitted BYMV^N at rates comparable with those of *M. persicae* and *A. pisum*. Transmission of BYMV^N also occurred with two other *N. bakeri* colonies maintained for 4 mo on *Trifolium pratense* L. (NZwc Sch 3B and Sch 7C) BYMV^N source plants. Each of these four BYMV^N transmission successes also demonstrated an unprecedented once-only transmission of BYMV^N by *N. bakeri* colonies. Our experience with western Oregon *N. bakeri* colonies was compared with descriptions of this native North American species after its 1960–1980s arrival in France, Germany, and Italy.

KEY WORDS vector, BYMV pathotypes, hypersusceptibility, *Trifolium* spp., epidemiology

THE EARLIEST REPORT THAT aphid vectors were transmitting Bean yellow mosaic potyvirus (family *Potyviridae*, genus *Potyvirus*, BYMV) in western Oregon was that of Crumb and McWhorter (1948); with definitive reports by Swenson (1954, 1958). BYMV is one of 86 viral species currently accepted as members of the genus *Potyvirus*, with 86 others tentatively accepted as members of this genus (van Regenmortel et al. 2000). Robertson and Klostermeyer (1961) described the dissemination of bean viruses (including BYMV) in central Washington; the scope of their studies was broadened by Hampton (1967), Landis and Hagel (1969), and Hagel and Hampton (1970). By 1962, 21 aphid species had been reported as vectors of BYMV (Kennedy et al. 1962), including the four species evaluated in this study. *Nearctaphis bakeri* (Cowen), an aphid native to North America, was first reported as a vector of a severe, but not necrosis-inducing, BYMV pathotype by Manglitz and Kreitlow (1960). *N. bakeri* was known to occur in Europe by the 1960s (Leclant 1967, Thieme 1991), and although characterized as a damaging pest of clover (Patti and Ricci 1979, Thieme 1991), it was not reported as a virus vector in Europe. Aberrant traits of BYMV-vectoring aphid species were reported by Sohi and Swenson (1964), Swain et al. (1964), and Kamm (1969), whereas limiting factors and aphid–vector behavioral traits were reported by Adlerz (1959).

The BYMV pathotypes inducing whole plant necrosis in bean, *Phaseolus vulgaris* L., cultivars have been variously referred to as inducing sudden death, bean necrosis, or top necrosis. The terms of choice for this article are BYMV^N, for necrosis-inducing bean yellow mosaic, which is principally reported herein, and BYMV^S, for the standard (mosaic-inducing) or BYMV type strain. Most *P. vulgaris* genotypes not bred and selected for resistance to BYMV^N are severely damaged by this pathotype (Baggett 1957, Tatchell et al. 1985), particularly Blue Lake-related cultivars (McWhorter and Frazier, 1950). In host range and pathology comparisons among 16 candidate isolates of BYMV, Jones (1974) and Jones and Diachun (1976) grouped nine, including BL-BNV and Gil-6 from western Oregon, which were representative of necrosis-inducing (severe) isolates of BYMV, e.g., BYMV^N. Of the remaining seven, two isolates were representative of BYMV^S type isolates, and five represented weakly pathogenic (mild) strains. Serological comparisons

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among 15 of these isolates agreed perfectly with pathotype groupings (i.e., OH isolate OH-Sb was compared with 15 other BYMV isolates by host reactions). This basic information effectively classified both BYMV pathotypes and serotypes, irrespective of plant source or vague terminology, and remains valid today.

BYMV-susceptible clover species grown in western Oregon for seed production or for mixed pasture have included alsike clover, *Trifolium hybridum* L.; crimson clover, *Trifolium incarnatum* L.; red clover, *Trifolium pratense* L.; New Zealand white clover, *Trifolium repens* L.; and subterranean clover, *Trifolium subterraneum* L. Others, sometimes existing as weed or volunteer plants, include old-field clover, *Trifolium arvense* L.; hop clover, *Trifolium dubium* Sibth.; and low hop clover, *Trifolium procumbens* L. Common vetch, *Vicia sativa* L., is also a host of legume viruses, including BYMV, and volunteer plants are abundant in western Oregon. $BYMV^N$ has most often been associated with white, alsike, and subterranean clovers, whereas $BYMV^S$ is more often associated with red clover (Hampton 1967, Hagel and Hampton 1970) and crimson clover (R.O.H., unpublished) in the Pacific Northwest. Zaumeyer and Thomas (1947) also considered white sweet clover, *Melilotus albus* Medikus, abundant in the Pacific Northwest, as an important host of $BYMV^S$. We also have isolated $BYMV^S$ from yellow sweet clover, *M. officianalis* Lam. Extensive plantings of *Gladiolus* in western Oregon also were reported to be a reservoir of aphid-transmitted BYMV (McWhorter et al., 1947) that was later found to contain necrosis-inducing forms of BYMV (F. P. McWhorter, unpublished). McWhorter and Frazier (1950) reported a devastating sudden-death disease of Blue Lake snap beans in western Oregon. The necrosis-inducing causal agent, $BYMV^N$, was later referred to as a "severe type strain" of BYMV (Tatchell et al. 1985).

Our purposes in this study were to establish the principal reservoir(s) of $BYMV^N$ and to identify aphid species associated with its spread. We present patterns of $BYMV^N$ (and $BYMV^S$) dissemination from clover, *Trifolium* spp., to proximal snap bean fields, *P. vulgaris;* aphid species associated with the spread of this virus; and laboratory trials of $BYMV^N$ transmission by four selected aphid species.

Materials and Methods

Study Sites. Two representative study sites were initially selected from our surveys of western Oregon snap bean fields being damaged by bean necrosis. At these sites, snap beans had been planted within 10 m of well-established clover fields, and percentage of incidence of virus-infected plants was determined for each 6.1-m row-transect (segment). Because virus dissemination had already occurred at study sites 1 and 2, associated vectors could not be determined. At study site 1, snap beans had been planted adjacent to a 4-yr-old stand of New Zealand white clover (NZwc). At study site 2, snap beans were planted adjacent to an older stand of mixed clovers, including both *T. repens* and *T. pratense.* Data points at each study site represent infected-plant percentages between two distances from clover, e.g., 4% incidence of $BYMV^N$ between 43 and 49 m (Fig. 1A). At all sites, clover and bean tissue samples were taken for virus identification assays. Symptoms were recorded, and buffered plant tissue extracts were tested by Ouchterlony agar double diffusion serology (Ball 1990) against antisera that reacted specifically to BYMV. Samples also were examined by thin-section electron microscopy and by electron photomicrographs of plant sap preparations (Hampton 1982). We also tested for contaminating viruses in laboratory inoculum source plants by indicator plant assays and electron microscopy.

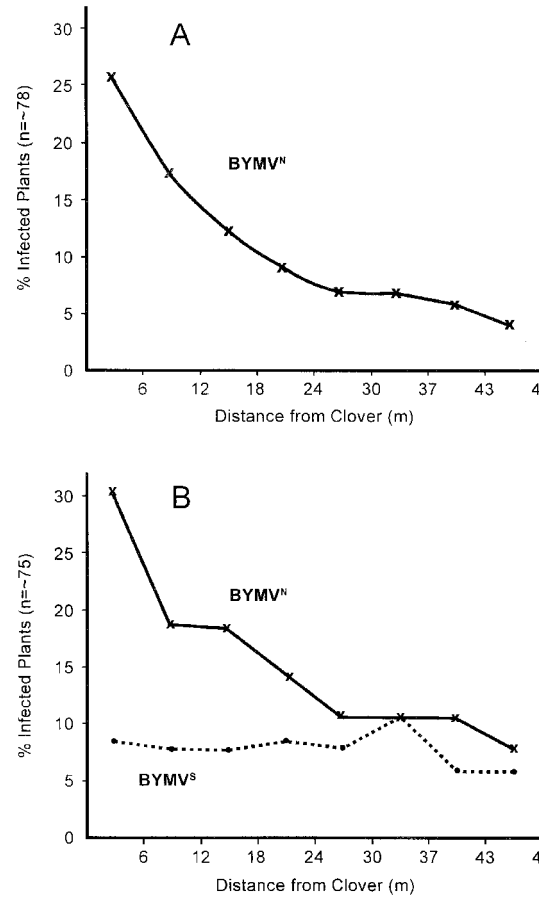

Fig. 1. Patterns of bean yellow mosaic potyvirus spread by aphids, from clover to snap beans, in western Oregon. (A) Study site 1, where only $BYMV^N$ was aphid disseminated. (B) Study site 2, where both $BYMV^N$ and $BYMV^S$ (pattern chlorosis-inducing pathotype) were distinctively disseminated.

Establishing Study Site 3. After the first season of this study, a unique site was brought to our attention, where bean plantings had consistently sustained damage by "bean necrosis." The grower expressed interest in our work, hoping to see the problem resolved. He anticipated planting bush Blue Lake beans the next year, and he took us to the projected planting site. To

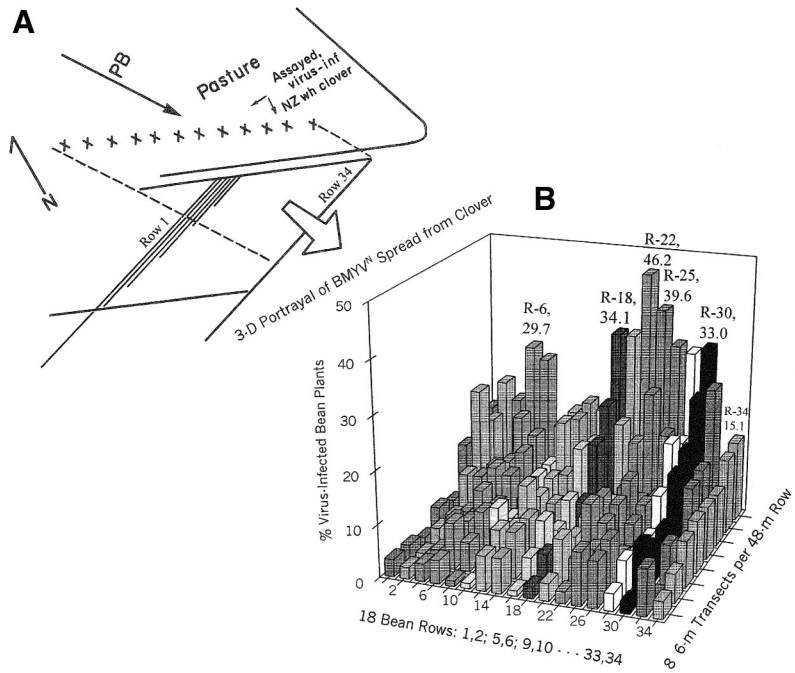

Fig. 2. Factors at study site 3 accompanying spread of BYMV^N from New Zealand white clover (NZwc) to OSU 58 (Blue Lake) bush-type snap-bean plants. (A) N, north; PB, prevailing breeze north-northwest. Xs represent the alignment of thousands of NZwc plants bordering the bean field. All 12 of the randomly selected NZwc plants contained serologically detectable BYMV^N. Aphid alatae migrating from clover to beans were collected and identified (see Table 1). (B) Infection rates among eight 6-m row-transects (≈90 plants) for each of 18 rows of bean plants. The distance across 34 rows was 30.5 m.

prepare for the study, we mapped the area and received permission to test NZwc plants located near the projected bean field and to remove BYMV^N-infected plants (all 12 of the 12 tested) for laboratory inoculum sources. The bean field had been planted by early June of the following year, and aphid flights had begun to reach bean plants 12 d after seedling emergence. The site was visited twice per week until no additional BYMV^N-infected plants were observable. A representative plot was then established, comprising 34 bean rows 48.8 m in length and 30.8 m in width (Fig. 2A). The numbers of infected plants were recorded for each of eight 6.1-m row-transects per 18 of the 34 rows. Note (Fig. 2B, front horizontal axis) that the 18 rows comprised alternate sets of two adjacent rows: 1, 2; 5, 6; 9, 10, and so on The average number of bean plants per 6.1-m transect was 91.3 ± 2.

Collection, Identification, and Maintenance of Aphid Species. By prior arrangement, aphid alatae arriving on bean leaves at site 3 in July were collected, preserved in 70% ethanol, and submitted for identification (Table 1) to Dr. V. F. Eastop (Department of Entomology, The Natural History Museum, London, United Kingdom). Four of the species known to feed on bean plants, clover spp., or a combination in western Oregon—*Myzus persicae* (Sulzer), *Acyrthosiphon pisum* (Harris), *Aphis fabae* Scopoli, and *Nearctaphis bakeri* (Cowen)—were designated for evaluation as BYMV^N vectors. The first three had been established as principal vectors of BYMV in western Oregon by Swenson (1958) and Sohi and Swenson (1964). *N. bakeri*, the majority species collected (Table 1), was first described by Cowen (1895), was early reported by Davis (1908) and Gillette (1908), and documented in Oregon by Leonard (1960).

Table 1. Alate aphid species collected (≈9 a.m.) from bean leaves at study site 3 coincident with the spread of BYMV^N from an adjacent pasture containing BYMV^N-infected New Zealand white clover

Aphid species[a]	No. collected	Proportion of total (%)
Acyrthosiphon pisum (Harris)	10[b]	4.6
Aphis fabae Scopoli[c]	29[b]	13.2
Aphis sp.	2	0.9
Brachycaudus helichrysi (Kaltenbach)	3	1.4
Brevicoryne brassicae (L.)	2	0.9
Capitophorus elaeagni (del Guercio)	2	0.9
Cavariella aegopodii (Scopoli)	1	0.5
Euceraphis sp.	1	0.5
Hyalopterus pruni (Geoffroy)	1	0.5
Hayhurstia atriplicis (L.)	1	0.5
Lipaphis erysimi (Kaltenbach)	2	0.9
Macrosiphum sp.	3	1.4
Metopolophium dirhodum (Walker)	2	0.9
Myzus persicae (Sulzer)	6[b]	2.7
Nearctaphis bakeri (Cowen)	149[b]	68.0
Sitobion avenae (F.)	5	2.3
Total collected	219	

[a] Identified by V. F. Eastop (Department of Entomology, Natural History Museum, London, United Kingdom).
[b] Species selected for BYMV^N vector studies.
[c] Only *A. fabae* was observed colonizing bean plants.

Table 2. Transmission of bean yellow mosaic virus (BYMV^N) by three known virus vector species, under controlled conditions

Aphid species	Trial no.	Fasting period (h)	Acquisition access period	Source plant[a]	Virus indicator plant[b]	Inoculation access time	Aphids/ plant	No. of plants infected	% transmission
M. persicae									
	1	15	10 min	RK bean	Faba	15 h	4	17/35	49
	2	15	10 min	RK bean	RK bean	20 h	2	2/29	7
	3	15	10 min	RK bean	RK bean	15 h	4	11/26	42
	4	15	1 h	Faba[c]	Faba	15 h	4	5/28	28
	5	15	3 h	RK bean	RK bean	15 h	10	6/11	54
	6	15	4 h	RK bean	Faba	15 h	2	5/22	23
	7	Reared on infected faba bean[c]			Faba	15 h	4	10/32	31
	Avg transmission rates, among all variables							56/183	30.6
A. pisum	1	15	3 min	RK bean	BT bean	90 min	3	15/38	39
	2	15	3 min	Alsike[d]	Faba	90 min	3	9/19	47
	Avg transmission rates, among variables							24/57	42
A. fabae	1	8	3 min	RK bean	BT bean	15 h	2	1/48	2
	2	6	5 min	Faba	Faba	15 h	4	2/52	4
	3	8	5 min	Alsike	RK bean	15 h	3	1/50	2
	4	8	10 min	Alsike	RK bean	15 h	3	1/43	2
	5	8	15 min	Alsike	BT bean	15 h	4	1/38	3
	Avg transmission rates, among all variables							6/231	2.6

[a] The BYMV^N-infected plant on which aphids were allowed access. Alsike white clover is a host of BYMV^N in western Oregon.
[b] Healthy plant seedlings to which viruliferous aphids were transferred, respectively, red kidney bean, faba bean, and black turtle (BT) bean (*P. vulgaris* L.).
[c] BYMV^N-inoculated faba bean was used as virus source plants only before their development of leaf necrosis.
[d] BYMV^N-inoculated alsike white clover plants were tested as alternate virus sources for *A. pisum* and *A. fabae.*

Subsequent to identification, colonies of each species were established, and wherever plausible, derived from alatae found on bean plant leaves. However, *N. bakeri* apterae were more readily collectable from NZwc plants adjacent to study site 3. After successive passages through virus-free host plants, colonies of *M. persicae*, *A. pisum*, and *A. fabae* were maintained in screened cages on virus-free red kidney (RK) bean, *P. vulgaris*, or faba bean, *Vicia faba* L., plants. *N. bakeri* colonies were likewise processed but maintained on virus-free *T. repens.* Each aphid species was tested under glasshouse (insectary) conditions for its capacity to vector BYMV^N. Repeated collections of *A. pisum* colonies were required because of their variable capacities to transmit BYMV^N, as also was reported by Kamm (1969). Similar problems occurred with colonies of *N. bakeri*, eventually requiring additional tests with colonies from a fourth site, in the third year of testing.

Source Plants. BYMV^N-infected *T. repens* transplants (NZwc/Gilbert #1–#12) from study site 3 were used as virus sources for mechanically inoculating RK bean and faba bean virus test plants with buffered extracts from macerated *T. repens* leaves. Infected bean and faba bean plants were then used as virus acquisition access plants for BYMV^N transmission by colonies of *M. persicae*, *A. pisum*, and *A. fabae* (Table 2). BYMV^N-inoculated alsike clover, *Trifolium hybridum*, plants were also used as virus sources for *A. pisum* and *A. fabae.* Continuing BYMV^N-induced symptoms on RK bean and faba bean also ensured opportunities to observe necrosis-inducing-pathotype stability throughout the study.

Insectary Transmission Procedures. Aphids were collected from virus-free maintenance plants, counted, placed into plastic vials, and fasted for 6–15 h before being placed onto BYMV^N-infected source plants (Tables 2 and 3). After acquisition access on infected plants for 3 min to 4 h (Day and Irzykiewicz 1954), aphids were placed onto seedlings of RK bean or faba bean plants. In each case, virus source plants and inoculated plants were then fumigated to eliminate any aphids surviving on them. Such routine procedures usually facilitated BYMV^N transmission by colonies of *M. persicae*, *A. pisum*, and *A. fabae* (Table 2). Although acquisition access periods of 3–5 min were usually sufficient for nonpersistently transmitted viruses, periods of acquisition access to infected plants were varied substantially for *M. persicae* (Table 2), because of preliminary results.

Delayed BYMV^N Transmission by *N. bakeri*. The unexpected incidence of BYMV^N transmission by *N. bakeri* clone-2 (C-2) and C-3 colonies demanded further investigation, particularly to ascertain minimal acquisition access periods for BYMV^N transmission. Accordingly, in July of the third year of trials, we obtained access to a fourth site where a bean crop, adjacent to an established mixed clover pasture, had been destroyed by BYMV^N. From that site, we obtained *N. bakeri* colonies from red clover, designated RC-1 (Table 3), and removed leaf samples from marked NZwc plants for serological and infectivity assays. Subsequently, four of these plants—Schellenberg (Sch) 3B, 4B, 7C, and 8A—were selected as inoculum sources. While transplanting these into the insectary, Sch 3B and Sch 7C were subdivided into clones-1 and -2 to compare periods of *N. bakeri* access to identical inoculum sources. RC-1 feeding periods on these BYMV^N-infected plants made up 1) continuous 4-mo access on clone-2, respectively, of Sch 3B

Table 3. Bean yellow mosaic virus (BYMV^N) transmission by *N. bakeri*: multiformities and protocol adjustments

Aphid colony	Trial no.	Fasting period (h)	Acquisition access time	Infected access plant	Virus indicator plant	Inoculation access time (h)	Aphids/ plant	No. of plants infected	% transmission
New Zealand white clover C-1[b]	1	15	1 h	NZwc #1	RK bean	15	6[a]	0/46	
	2	5	1 h	NZwc #4	RK bean	15	6	0/24	
	3	5	1 h	NZwc #6	RK bean	15	6	0/28	
	4	15	1 h	NZwc #11	RK bean	15	6	0/54	
New Zealand white clover C-2	1	15	5 h	NZwc #6	Faba	15	10	0/45	
	2	0	Lifetime[c]	NZwc #6[d]	RK bean	15	10	29/42	69.0
	3	0	Lifetime	NZwc #6	RK bean	15	10	0/54	
	4	0	Lifetime	NZwc #6	RK bean	15	10	0/50	
New Zealand white clover C-3	1	15	5 h	NZwc #11	Faba	15	10	0/45	
	2	0	Lifetime[c]	NZwc #11[d]	RK bean	15	10	18/42	42.9
	3	0	Lifetime	NZwc #11	RK bean	15	10	0/54	
	4	0	Lifetime	NZwc #11	RK bean	15	10	0/50	
Red clover RC-1	1a	15	3 × 5 h	NZwc Sch 3B[e]	RK bean	15	10	0/55	
	1b	0	4 mo	NZwc Sch 3B[f]	RK bean	15	10	1/9	11
	2a	15	3 × 5 h	NZwc Sch 7C[e]	RK bean	15	10	0/56	
	2b	0	4 mo	NZwc Sch 7C[f]	RK bean	15	10	2/5	40
	3	15	2 × 5 h	NZwc Sch 4B[g]	RK bean	15	10	0/17	
	4	15	1 × 5 h	NZwc Sch 8A[g]	RK bean	15	10	0/5	

[a] Number of aphids transferred to individual red kidney (RK) bean or faba bean assay plants, after timed access to BYMV^N-infected New Zealand white clover (NZwc) plants.

[b] C-1 represents three *N. bakeri* colonies derived from apterae taken from NZwc leaves at study site 3.

[c] *N. bakeri* colonies that emerged onto BYMV^N-infected NZwc plants #6 and #11, which had been transplanted from study site 3. After emergence, they were transferred directly to RK bean assay plants.

[d] Aphid colonies C-2 and C-3 lived continuously on BYMV^N-infected NZwc plants #6 and #11, respectively, before being tested for BYMV^N transmission. All successive transmission tests with the same colonies were negative.

[e] *N. bakeri* RC-1 colonies were allowed access to clone-1 three times, respectively, on NZwc Sch 3B and Sch 7C, and then transferred each time to RK bean BYMV^N test plants, for 15 h. 'Clone' signifies quasi-equal dissection of selected BYMV^N source plants, before transplanting.

[f] RC-1 colonies were maintained continuously for 4 months on clone-2 of Sch 3B and Sch 7C, respectively, and then transferred to RK bean BYMV^N test plants, i.e., transmissions from these clones (trials 1b and 2b) were associated with the continuous access to inoculum of 170 (10 by 17) *N. bakeri* apterae.

[g] RC-1 colonies were given access to plant Sch 4B twice and to plant Sch 8A once, for 5 h each, and then transferred to RK bean BYMV^N test plants.

and Sch 7C; 2) a series of three short-term (5-h) accesses to clone 1 of the same inoculum source; 3) a series of two 5-h accesses to Sch 4B; and 4) a single 5-h access to Sch 8A. After each of these access periods, RC-1 aphids were transferred to RK bean BYMV^N test plants. These treatments during July–November or December of the third year enabled us to observe possible effects of season on 1) virus acquisition and transmission capacity by RC-1 colonies, and 2) cyclically variable BYMV^N translocation and concentration in NZwc plants.

Glasshouse (insectary) temperatures during the time of BYMV^N transmissions by *N. bakeri* colonies (October–early December) ranged between ≈15 and 21°C. Supplemental overhead fluorescent illumination extended daylight length by 10 h and was placed within 30–50 cm of experimental plants.

Results and Discussion

Field Studies. Maximal BYMV^N infection rates at study sites 1–3 ranged from 26 to 46%, occurring consistently closest to clover/BYMV^N inoculum sources, representative of the nonpersistent transmission of BYMV. At study site 1 (Fig. 1A), BYMV^N was spread to bean plants at distances exceeding 49 m, declining from 26% incidence at 3 m from clover to 4% at 49 m from clover (percentage of infected plants between measured points). The 31% incidence of BYMV^N at study site 2 (Fig. 1B), where beans were planted adjacent to a pasture of mixed clover species, was similar to that at study site 1. In contrast, BYMV^S incidence varied only from 6 to 10% among the seven 6.1-m segments from clover. Because symptoms induced by the two BYMV pathotypes are clearly distinguishable (pattern chlorosis of leaves versus leaf and plant necrosis), the incidence of each pathotype was recorded within each 6.1-m transect of bean rows, from 3 to 49 m away from clover. No dual infections per plant were observable. Vectors of these coincidental pathotype disseminations (Fig. 1B) were not determined; however, red clover is more often infected by BYMV^S in western Oregon, whereas white clover and subterranean clover are more often infected by BYMV^N (R.O.H., unpublished).

In early July, 16 species of aphids (Table 1) were collected from bean leaves at study site 3 (Fig. 2A; Table 1). The zone between dashed lines was estimated as the bean field area most exposed to breeze-assisted aphid movements. Graphic infection rates are shown (back to front) at successively farther distances from infected clover plants. The highest infection rates across the rows (Fig. 2B, tall bars), viewed left to right, consistently occurred in the 6.1-m row-transects closest to clover, e.g., 29.7% (row 6), 34.1%, (rows 18 and 21), 46.2% (row 22), 39.6% (row 25), 33.0% (row 30), and 15.1% (row 34). Infection rates in these transects closest to clover (rows 1–34) represented an

arched, skew-shaped curve, suggesting landing and probing patterns of BYMV^N vectors. Exceptionally, only 8.8% of the plants in the closest transect of row 33 were infected (obscured in Fig. 2B). Within the 49-m length of row 22, the percentage of infected plants per transect decreased from 46.2 to 2.2%, again typifying short-distance virus spread patterns by viruliferous aphids. Infection rates in transects farthest from clover (graphically, closest to the viewer) ranged irregularly from 1 to 10%, in contrast with bean plant transects closest to the NZwc plants.

Laboratory Studies. Standardized acquisition access periods (Table 2) sufficiently effected transmission of BYMV^N by three of the four aphid species, including rearing on infected plants (*M. persicae*, trial 7). Neither the kinds of infected plants on which they were provided access, nor plants to which they were transferred, nor the time allowed on test plants influenced rates of virus transmission. *M. persicae* and selected colonies of *A. pisum* were comparable in vector efficiency, whereas *A. fabae,* the only species colonizing bean plants in this study, was consistently and substantially less effective in vectoring BYMV^N under laboratory conditions (Table 2). This difference from *M. persicae* or *A. pisum* is consistent with dependence of *A. fabae* (as a colonizing species) on phloem feeding, where virions are substantially less concentrated than in leaf parenchyma cells.

We experienced an occasional, retrospectively significant behavior pattern of *N. bakeri* colonies during the course of this study. In most cases, after fasting, aphids fed normally on leaves of both virus inoculum source plants and on virus assay hosts. On some occasions, however, each aphid that had been removed from virus-free NZwc rearing plants and placed onto a virus access plant (e.g., NZwc #1–#12) moved quickly and directly down the petiole to the base of the plant, repeating the behavior as often as placed back onto the leaves. This behavior, precluding BYMV^N transmission attempts, was later recognized as an instinctive attempt to escape into subterranean exules ("escape tunnels"), a trait elaborated below.

Colonies of *N. bakeri* (Table 3, C-1) failed to transmit BYMV^N by standardized methods during 32 test variations over 15 mo. Our testing persistence and methods were influenced by data published by Manglitz and Kreitlow (1960), in which a severe (but not necrosis-inducing) BYMV isolate was transmitted to Bountiful bean plants by *Anuraphis bakeri* (seq. *N. bakeri*) after 1-h access acquisition feeding on infected Ladino clover (*T. repens*). No such results were obtained during our 32 test variations with C-1 colonies of *N. bakeri.* It was confusing, therefore, when, in the 16th month of testing (October), colonies of *N. bakeri* emerged on NZwc transplants #6 (colony 2) and #11 (colony 3) and transmitted BYMV^N from these transplants at rates of 69 and 42.9%, respectively (Table 3). When our procedures were critically reviewed, it was considered improbable that *N. bakeri* could have survived sequential fumigations of NZwc virus source plants, not reused until new foliage had been generated. We neither expected nor observed residual presence of *N. bakeri* C-1 colonies during the initial 15 mo of testing BYMV^N transmission by this species. Indeed, there had been every reason to assume that aphid transmissible potyviruses would be transmitted nonpersistently; thus, longer access feeding periods or other parameters were not tested for colonies of C-1 (Table 2).

Transmission rates of 69 and 42.9% were fully equivalent to those of *M. persicae* and *A. pisum*, but with two fundamental transmission restrictions of a potyvirus by our *N. bakeri* colonies: 1) prerequisite long-term *N. bakeri* acquisition access feeding on BYMV^N-infected NZwc transplants, and 2) *N. bakeri* transmission from infected NZwc transplants one time only (Table 3, colonies C-2 and C-3, trials 3 and 4, respectively). It was thus obvious that, if these results were verifiable, the vector–virus attributes would be unprecedented for aphid access of a stylet-borne virus.

To further probe the nature of BYMV^N transmission by *N. bakeri* colonies C-2 and C-3, new *N. bakeri* colonies and new NZwc sources of BYMV^N were established for additional tests, particularly to determine whether intermediate-term access to infected clover plants could facilitate transmission of BYMV^N. Thus, during a 4-mo period (late July–November/December), RC-1 colonies were provided four types of access among the four BYMV^N-infected NZwc transplants (Table 3): 1) three times, RC-1 was allowed access to clone #1 (Table 3, footnote e) of Sch 3B and of Sch 7C (Table 3, trials 1a and 2a); 2) RC-1 was allowed continuous access to clone-2 of these inoculum sources, for 4 mo (trials 1b and 2b); 3) RC-1 was allowed access to Sch 4B two times for 5 h (trial 3); and 4) RC-1 was allowed access to Sch 8A once for 5 h (trial 4). After each of these access periods, the aphids were placed directly onto RK bean BYMV^N test plants and left 15 h before fumigation. Results from these tests provided two examples in which continuous 4-mo access periods were associated with BYMV^N transmission (trials 1b and 2b), whereas singular or repeated short-term access feeding during the 4-mo period (trials 1a, 2a, 3, and 4) resulted in no transmissions. The number of test plants inoculated in trials 1b and 2b were limited by numbers of aphids available ($\approx$90 and $\approx$50, respectively) after 4-mo access to clone 2 NZwc virus source plants. We interpret the low transmission rates as either 1) unknown physiological or life cycle traits of *N. bakeri*, limiting or facilitating BYMV^N transmission, or both; or 2) fulfillment of a quasi-minimal (4-mo) time requirement for BYMV^N acquisition and transmission. Such "long-term periodicity" of BYMV^N transmission by *N. bakeri* (or species of *Anuraphis* and *Dysaphis*), combined with one-time-only transmission, is unprecedented for stylet-borne viruses and for their aphid vectors. Further examination of this trait may be more readily resolvable in the field than in the laboratory, i.e., direct transmission assays by *N. bakeri* immediately upon their emergence onto BYMV^N-infected white clover leaves, followed by timed intervals thereafter. In such studies, data on *N. bakeri* postemergence feeding behavior also should be determined (i.e., whether prob-

ing leaf phloem tissues or prolonged feeding in vascular tissues). Manglitz and Kreitlow (1960), reported no such prerequisites for BYMV transmission by *N. bakeri.* Instead, after a 1-h access of their *N. bakeri* (*Anuraphis bakeri*) colony to BYMV-infected Ladino white clover plants, the virus was repeatedly transmitted to Bountiful bean plants under insectary conditions, by four or more aphids per plant.

Based particularly on information provided by V. F. Eastop, we concluded that *N. bakeri* had emerged from soil or "otherwise concealed parts of (the) plants," which he indicated is also true for "the species of *Anuraphis* and *Dysaphis.*" He further explained that "The genus *Nearctaphis* (in North America) has subterranean exules" and that "the presence of the aphids is usually indicated by their attendant ants." Patti and Ricci (1979) also indicated that in central and southern Italy, "*N. bakeri* colonies were concentrated on the lower parts of (red clover) plant(s) in April–May" and that they "spread all over the plants during the population peak in May–June and descended below soil level during the hottest and driest weather in July–August." This appropriately describes our experience in the insectary with *N. bakeri* colonies C-2 and C-3 (Table 3), in the same environment reported by Thieme (1991), i.e., that *N. bakeri* was first "found on *Trifolium pratense* in a glasshouse at Rostock, Germany, in December 1989." And as similarly described by Patti and Ricci, our C-2 and C-3 colonies of *N. bakeri* were found "spread(ing) all over" $BYMV^N$-infected NZwc transplants, 15 mo after moving them to the insectary; plausibly having been sustained on crown and root tissues of NZwc plants (*T. repens*).

Obtaining our colonies of *N. bakeri* from infested red clover, for concluding $BYMV^N$ transmission tests, paralleled the first report of this aphid in Europe. Our results, however, suggested that $BYMV^N$-vectoring traits by *N. bakeri* were comparable, whether colonies had occupied *T. repense* or *T. pratense,* e.g., were characterized by long-term virus acquisition and one-time-only transmission.

Significant distinctions between North American and European observations included 1) European populations of *N. bakeri* were not reported as vectors of BYMV or other viruses, and 2) there was no indication of *N. bakeri*–ant symbionts, e.g., perhaps left behind in North America when *N. bakeri* was disseminated to Europe. Instead, *N. bakeri* in Europe is simply considered to be a destructive clover pest, including selections of Persian and Egyptian clover (Patti and Ricci 1979).

Information deserving further investigation by others include: 1) completed life cycles, morphs, and further behavioral traits of *N. bakeri,* including both North American (holocyclic) and European (anholocyclic) colonies, a la Thieme (1991); 2) evaluations of white and red clover leaf, crown, and root tissues as *N. bakeri* food sources, encompassing the life cycle context; and for North American *N. bakeri* particularly, because $BYMV^N$ transmission by this species was established herein; 3) determine variations among *N. bakeri* colonies or biotypes for their capacity to transmit $BYMV^N$; 4) establish comparative $BYMV^N$ titers in each of the tissues mentioned above; and 5) quantify $BYMV^N$ transmission rates by *N. bakeri* when allowed access to these phloem and parenchyma tissues individually. Furthermore, the nature and properties (including morphs) of *N. bakeri* transmission of $BYMV^N$ under field conditions should be compared directly with those observed in our laboratory, e.g., is *N. bakeri* capable of transmitting the virus immediately upon emergence from underground exules, or does it acquire virus only after emergence, by feeding directly on infected NZwc leaves? If only by the latter, is transmission associated specifically with singular or successive probes, only in leaf parenchyma? Finally, is the "one-time-only $BYMV^N$ transmission" phenomenon demonstrable under both laboratory and field conditions?

In summary, no virus transmission occurred after singular or repeated 5-h accesses of RC-1 to selected $BYMV^N$ inoculum sources. Transmission by RC-1 colonies occurred only when maintained 4 mo on clones 2 of Sch 3B and Sch 7C. Three of 17 assay plants became infected, after an apparently minimal 4-mo-long *N. bakeri* access to these NZwc inoculum sources, i.e., three of 14 sets of 10 aphids (≈16%) transmitted $BYMV^N$, indicating that these aphids had fed on virus-infected source plants long enough to facilitate $BYMV^N$ transmission. On a larger scale, 47 sets of 10 *N. bakeri* (colonies C-2 plus C-3) transmitted $BYMV^N$ to 84 RK test plants (≈56%). These separate, contrasting results may only represent estimates of minimal and optimal acquisition access periods for *N. bakeri* transmission of $BYMV^N$. In any case, these results increase the probability that *N. bakeri,* making up 68% of the aphid species disseminated from NZwc to snap bean plants (Table 1), was a major vector of $BYMV^N$ in these studies.

The distinction between our results with *N. bakeri* and those of Manglitz and Kreitlow (1960) suggests that there are North American variants of this species, perhaps comprising diverse biotypes, some of which are not symbiotic with ants; and as such, capable of directly transmitting BYMV pathotypes. Similarly, the *N. bakeri* traits discussed above may be tied specifically to ant–symbiont colonies. And if so, other scientists may have an opportunity to determine how and why such symbiosis facilitates or modifies *N. bakeri* capability for vectoring $BYMV^N$. The nature of our results with ant-symbiotic colonies of *N. bakeri* are unrelated to those described for other aphid species by Sohi and Swenson (1964), Swain et al. (1964), and Kamm (1969), in which virus-vectoring capacity was diverse within individual species, as experienced by us among tested or selected colonies of *A. pisum.*

It is not impossible that, in our studies, *N. bakeri* could have emerged without being observed and individuals were thus the functional vectors in $BYMV^N$ transmission from Sch 3B or Sch 7C (trials 1b and 2b). However, emergent aphids would have been more readily observable in virus source plants involved in trials 1a, 2a, 3, and 4, each having been fumigated after each 5-h hosting of *N. bakeri.* And, in no case, were

emergent aphids observed on these virus source plants. Transmission failures by RC-1 colonies after trials 1b and 2b (not shown) also substantiated the one-time-only aphid vector transmission of a potyvirus, the phenomenon first manifested by *N. bakeri* colonies C-2 and C-3.

Desiccated, purified $BYMV^N$ inoculum sources can be provided to scientists wishing to further investigate $BYMV^N$–vector–host relationships. Requests can be made via hamporc@fmtc.com.

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